EXCYSTATION OF APOSTOME CILIATES IN RELATION TO MOLTING OF THEIR CRUSTACEAN HOSTS

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The dependence of certain aspects of the life cycle of a parasite on certain physiological activities of its host is a common phenomenon. One need only mention, as examples, the synchronicity of reproduction in malaria parasites, which is dependent on the diurnal rhythm of activity of the host (Stauber, 1939), and the appearance of sexual reproduction in the intestinal protozoa of the roach *Cryptocercus,* which is provoked by the molting of the host (Cleveland and Nutting, 1955). In a similar way, some species of apostome ciliates exist as small cysts (phoronts) on the gills of various Crustacea and excyst only at the time when the host molts (Chatton and Lwoff, 1935). The excysted forms (trophonts) then engorge rapidly on the proteinaceous fluid in the shed skin of the host. The much larger organisms so produced form free-living cysts. Within these cysts a series of divisions occurs which results in the formation of a number of small daughter ciliates (tomites). These swim about, apparently without feeding, until they have been drawn into the gill chamber of a suitable crustacean host. Here they encyst on the gills and again remain quiescent until the new host molts. The entire cycle depends on a single meal obtained from the molting fluids of the host, and the molting of the host provides the only stimulus to excystation (Chatton and Lwoff, 1935).

It seemed of interest to attempt to produce excystation *in vitro.*

Materials and Methods

Two host-parasite combinations have been used: (1) the fiddler crab *Uca pugnax* and a probably undescribed species of *Gymnodinioides;* (2) the hermit crab *Pagurus longicarpus* and *Gymnodinioides inkystans.* The fiddler crabs were kept in dishes with a shallow layer of sea water which was changed daily. Isolated individuals were kept in paper cups with a little sea water and a screen cover. The hermit crabs were maintained in running sea water. Isolated crabs of this species were placed in 100-ml. beakers covered with a piece of gauze held on by a rubber band. The beakers were immersed in an aquarium of running sea water. Both species were fed pieces of clam once or twice a week.

Individuals of *Uca pugnax* which were near the molt were generally recognizable by a peculiar pale cast to the carapace and legs. Those in the process of molting were observed to have milky white blood. In order to identify crabs very near the molt, the tip of a leg was cut off to permit a drop of blood to exude. The blood had a clear appearance except just before molting, when it was milky.

Crabs of the species *Pagurus longicarpus* near molting could be recognized by a marked gray color of the carapace and legs, as distinguished from the reddish cast

of individuals which had recently molted. In small groups of isolated "gray" crabs about 50% molted within three days after isolation, whereas none molted in corresponding groups of "red" crabs.

For the *in vitro* experiments, sterile Petri dishes holding two discs of filter paper and one or two depression slides were used. The filter paper was moistened with sterile sea water. In the concavity of each depression slide was placed a droplet of sterile sea water containing, per ml., 500 or 1000 units of penicillin G and 0.5 or 1 mg. of streptomycin. The lower concentrations were used in the experiments with *Uca* and the higher concentrations in the experiments with *Pagurus*. A crab selected to serve as a source of infected gill material was immersed briefly in 70% ethanol, rinsed in sterile sea water, and blotted on sterile filter paper. The legs were cut off close to the body with sterile scissors and the blood allowed to exude into the droplet of sea water with antibiotics. Two such blood-sea water mixtures could be prepared from one crab. In the experiments with *Uca* the blood was diluted by the sea water about 1 : 1, in those with *Pagurus* about 1 : 3. The carapace of the crab was then torn off. At this step, if the animal was close to molting, the old carapace cuticle would readily come loose revealing the soft newly formed skin beneath it. After exposure of the gill chamber, the gills were plucked off and placed in the mixtures of blood and sea water with antibiotics.

The preparations were kept at room temperatures in dim light and observed with a dissecting microscope for the appearance of trophonts. The gills were later placed between slide and coverslip and examined for the presence of phoronts and for possible signs of excystation.

Results

A. *Observations*

1. *Gymnodinioides* sp. of *Uca pugnax*. About 80% of the crabs of this species examined showed a few to many phoronts on their gills. The incidence of trophonts was, however, much lower. Out of a series of 105 recently shed skins only 24 contained trophonts. This might have been the result of very rapid engorgement and early escape of the trophonts from the exuviae, especially since molting usually occurred during the night. On the other hand, it might be that *Uca* is a relatively unfavorable host. The phoronts were frequently surrounded by a cellular reaction, often containing considerable brown pigment. Such a host reaction was never seen in *Pagurus*.

The living trophonts of this ciliate had a more pointed and twisted posterior end than those of *Gymnodinioides inkystans* from *Pagurus*. A silver preparation suggested 10 or 11 rather than 9 ciliary bands. The developmental cycle was typical of the genus *Gymnodinioides* (Chatton and Lwoff, 1935). More detailed morphological study would be needed for the precise identification of this organism, which does not quite fit any of the described species of *Gymnodinioides*.

In gill cuticle material taken from crabs found in the act of molting, small trophonts which had already excysted were present, as well as cysts showing a motile trophont within, and also seemingly unchanged resting phoronts. Cysts containing a motile trophont did not show the conspicuous large refractile granules present in most of the other phoronts. This might indicate a utilization of reserve food granules during the encysted state, a suggestion already made by Miyashita

(1933). The following observation on re-infection, however, is not entirely in accordance with this idea.

One *Uca* isolated in a small dish with a little sea water molted during the night. In the morning engorged trophonts were found swimming about in the dish. The crab was removed to a separate dish. By the following day cysts undergoing tomite formation were observed in the first dish and the crab was returned to it. The next day active tomites were noted in the water of this dish. One day later (three days after the molt) the crab was killed and its gills examined. Eighteen phoronts were found. All of these had few reserve granules and most had a large vacuole near the posterior end. In several this vacuole was seen to collapse and re-form slowly. Had these cysts not been found on a host which was known to have just molted and just been re-infected, they might have been considered "ripe" phoronts ready to excyst.

2. *Gymnodinioides inkystans* of *Pagurus longicarpus*. At least 80% of the molt skins of *Pagurus* examined during three summers contained moderate to large numbers of trophonts which were clearly *G. inkystans*. Phoronts were found on the plicae of the gills of most of the hermit crabs examined.

B. *Experiments in vitro*

1. *Gymnodinioides* sp. of *U. pugnax*. In an initial experiment gills from a crab in the act of molting were placed in a droplet of sea water and in sea water containing a segment of leg integument. Active small trophonts were already present in the gills, but nowhere else, at the time of the preparation. Eight hours later only small trophonts were seen in the preparation with sea water alone, but in the other preparation numerous partially to fully engorged trophonts were swimming about. A few were already encysting. By the second day many active tomites had been formed. Thus, once excystation had occurred, the engorgement and subsequent development *in vitro* were essentially normal.

In all the later experiments, with both host-parasite combinations, the methods detailed in the section on Materials and Methods were used. The general plan was to pair a crab judged to be not near the molt with one considered close to molting. The gills of each were divided into two portions. One portion was placed in a droplet of sea water with antibiotics plus blood of the same crab, and the second portion in a similar droplet with blood of the other crab.

Out of five experiments of this type in which numerous phoronts were present on the gills of both crabs, fully formed trophonts ready to excyst and showing slight movements were found in three. In these three experiments the crab judged to be near molting had milky blood, whereas in the other two experiments the crab considered near molting had clear or faintly turbid blood. In two of the three positive experiments the signs of excystation occurred only on gills of the crab near molting in its own blood. In the third, they occurred on gills of the crab near molting in the blood of the non-molting one. In no case did phoronts from the gills of the non-molting crab show signs of excysting.

2. *Gymnodinioides inkystans* of *P. longicarpus*. Seven experiments of the paired type were done in which phoronts were present on the gills of both the non-molting crab and the crab near molting. The following observations were made.

Exp. 1. Seven hours after placing of the gills in the blood-sea water-antibiotics mixture, four large trophonts were seen in the preparations containing gills from the crab near molting in its own blood, and one small trophont in the preparation of gills from this same crab in the blood of the non-molting crab.

Exp. 2. No trophonts were seen after seven hours, but after one day one large trophont was present in the preparation of gills from the crab near molting in its own blood, and two large trophonts in the preparation of gills from the crab near molting in the blood of the non-molting crab.

When the gills of the non-molting crab in its own blood were then examined under higher magnification, the unexpected observation was made of three phoronts which showed ciliary movement within the cyst (out of a total of 16 seen). Of 20 phoronts seen on the gills of the non-molting crab in blood of the one near molting, none showed movement although three had a large vacuole near the posterior end.

Exp. 3. A large trophont was present after one day in the gills from the crab near molting held with its own blood. None of the other preparations showed any indication of excystation.

Exp. 4. After one day four trophonts had developed from the gills of the crab near molting held with its own blood, and one from the gills of this crab with blood of the non-molting crab.

Exp. 5. Within seven hours six trophonts had developed from the gills of the crab near molting in its own blood, and one trophont from the gills of this crab in blood of the non-molting one. By the next day two additional trophonts had appeared in the latter preparation.

Exp. 6. Six trophonts developed within seven hours from the gills of the crab near molting in blood of the non-molting one. Partial drying had occurred in the preparation containing gills of the crab near molting in its own blood.

Exp. 7. No trophonts or signs of excystation could be found in any of the preparations.

Discussion

Excystation of both species of *Gymnodinioides* can evidently take place *in vitro* from phoronts which have not yet begun to excyst *in vivo*. The host crustacean must, however, be very close to molting time for this to occur, so that the statement of Chatton and Lwoff (1935) that these phoronts excyst only in response to molting of their host still holds. Phoronts from the gills of crabs near molting excysted, on the whole, a little better in the presence of the homologous blood than in the presence of blood from a non-molting crab. Miyashita (1933) noted that certain "ripe" cysts of *G. caridinae* from a fresh-water shrimp excysted within a few minutes after being placed under a coverslip in body fluid of the host, whereas many "unripe" cysts did not. It might be that the successful excystation occurred only with cysts which happened to have been taken from a shrimp near molting.

The results of the experiments described in the present paper indicate that the encysted phoronts of *Gymnodinioides* may require a series of stimuli from the host in order to prepare them for the final stimulus which produces excystation just before the actual molt. Such a course of events would be somewhat analogous to that described by Cleveland and Nutting (1955) for the sexual phenomena of the protozoa of *Cryptocercus*. Protozoa transferred from a roach at one stage of the

molting cycle to another at a different stage invariably died, whereas those transferred to another roach at the same stage continued their development.

Summary

Observations and experiments have been made with the encysted phoronts of *Gymnodinioides inkystans* on the gills of the hermit crab (*Pagurus longicarpus*) and of *Gymnodinioides* sp. on the gills of the fiddler crab (*Uca pugnax*). The phoronts of both species would excyst *in vitro,* in a mixture of crab blood with sea water and antibiotics, and give rise to engorged trophonts, only if the cysts were taken from gills of a crab which was near molting. This excystation occurred somewhat more readily in the presence of blood from the same crab, near the molt, than in the presence of blood from a crab not close to molting. It is concluded that the encysted phoronts probably require a series of stimuli from the host in order to prepare them for the final stimulus which produces excystation just before the actual molt.

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